FORM-PTO-1390 (Rev. 12-29-99) U.S. DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE

TRANSMITTAL LETTER TO THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US) CONCERNING A FILING UNDER 35 U.S.C. 371

ATTORNEY'S DOCKET NUMBER: 016800-464

U.S. APPLICATION NO. (If known, see 37 C.F.R. 1 5): UNASSIGNED 09/937320

INTERNATIONAL APPLICATION NO.	INTERNATIONAL FILING DATE	PRIORITY DATE CLAIMED
PCT/FR00/00561	7 MARCH 2000	24 MARCH 1999

TITLE OF INVENTION: USE OF VITAMIN C OR THE LIKE FOR STIMULATING SKIN CELL SYNTHESIS

APPLICANT(S) FOR DO/EO/US: Andre ROUGIER et al.

Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information:

1. ☒ This is a **FIRST** submission of items concerning a filing under 35 U.S.C. 371.
2. ☐ This is a **SECOND** or **SUBSEQUENT** submission of items concerning a filing under 35 U.S.C. 371.
3. ☒ This is an express request to begin national examination procedures (35 U.S.C. 371(f)) at any time rather than delay examination until the expiration of the applicable time limit set in 35 U.S.C. 371(b) and the PCT Articles 22 and 39(1).
4. ☒ A proper Demand for International Preliminary Examination was made by the 19th month from the earliest claimed priority date.
5. ☒ A copy of the International Application as filed (35 U.S.C. 371(c)(2))
   a. ☒ is transmitted herewith (required only if not transmitted by the International Bureau).
   b. ☒ has been transmitted by the International Bureau.
   c. ☐ is not required, as the application was filed in the United States Receiving Office (RO/US)
6. ☒ A translation of the International Application into English (35 U.S.C. 371(c)(2)).
7. ☐ Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. 371(c)(3))
   a. ☐ are transmitted herewith (required only if not transmitted by the International Bureau).
   b. ☐ have been transmitted by the International Bureau.
   c. ☐ have not been made; however, the time limit for making such amendments has NOT expired.
   d. ☐ have not been made and will not be made.
8. ☐ A translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 371(c)(3)).
9. ☐ An oath or declaration of the inventor(s) (35 U.S.C. 371(c)(4)).
10. ☐ A translation of the annexes to the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371(c)(5)).

Items 11. to 16. below concern other document(s) or information included:

11. ☒ An Information Disclosure Statement under 37 CFR 1.97 and 1.98.
12. ☐ An assignment document for recording. A separate cover sheet in compliance with 37 CFR 3.28 and 3.31 is included.
13. ☒ A FIRST preliminary amendment.
    ☐ A SECOND or SUBSEQUENT preliminary amendment.
14. ☐ A substitute specification.
15. ☐ A change of power of attorney and/or address letter.
16. ☒ Other items or information:

Form PCT/IB/303; International Search Report; International Preliminary Examination Report; Form PCT/IB/332;

U.S. APPLICATION NO. (If known, see 37 CFR 1.5)	INTERNATIONAL APPLICATION NO.	ATTORNEY'S DOCKET NUMBER
UNASSIGNED 09/937320	PCT/FR00/00561	016800-464

17. ☐ The following fees are submitted:	CALCULATIONS	PTO USE ONLY
Basic National Fee (37 CFR 1.492(a)(1)-(5)):		
Neither international preliminary examination fee (37 CFR 1.482) nor international search fee (37 CFR 1.445(a)(2)) paid to USPTO and International Search Report not prepared by the EPO or JPO $1,000.00 (960)		
International preliminary examination fee (37 CFR 1.482) not paid to USPTO but International Search Report prepared by the EPO or JPO $860.00 (970)		
International preliminary examination fee (37 CFR 1.482) not paid to USPTO but international search fee (37 CFR 1.445(a)(2)) paid to USPTO $710.00 (958)		
International preliminary examination fee paid to USPTO (37 CFR 1.482) but all claims did not satisfy provisions of PCT Article 33(1)-(4) $690.00 (956)		
International preliminary examination fee paid to USPTO (37 CFR 1.482) and all claims satisfied provisions of PCT Article 33(1)-(4) $100.00 (962)		
ENTER APPROPRIATE BASIC FEE AMOUNT =	$ 860.00	
Surcharge of **$130.00 (154)** for furnishing the oath or declaration later than 20 ☐ 30 ☐ months from the earliest claimed priority date (37 CFR 1.492(e)).	$	

Claims	Number Filed	Number Extra	Rate		
Total Claims	12 -20 =	0	X$18.00 (966)	$ 0.00	
Independent Claims	4 -3 =	1	X$80.00 (964)	$ 80.00	
Multiple dependent claim(s) (if applicable)			+$270.00 (968)	$ 0.00	
TOTAL OF ABOVE CALCULATIONS =				$ 80.00	
Reduction for 1/2 for filing by small entity, if applicable (see below).				$	-
SUBTOTAL =				$ 940.00	
Processing fee of **$130.00 (156)** for furnishing the English translation later than 20 ☐ 30 ☐ months from the earliest claimed priority date (37 CFR 1.492(f)). +				$	
TOTAL NATIONAL FEE =				$ 940.00	
Fee for recording the enclosed assignment (37 CFR 1.21(h)). The assignment must be accompanied by an appropriate cover sheet (37 CFR 3.28, 3.31). **$40.00 (581)** per property +				$	
TOTAL FEES ENCLOSED =				$ 940.00	
				Amount to be: refunded	$
				charged	$

a. ☐ Small entity status is hereby claimed.

b. ☒ A check in the amount of $ 940.00 to cover the above fees is enclosed.

c. ☐ Please charge my Deposit Account No. 02-4800 in the amount of $_____ to cover the above fees. A duplicate copy of this sheet is enclosed.

d. ☒ The Commissioner is hereby authorized to charge any additional fees which may be required, or credit any overpayment to Deposit Account No. 02-4800. A duplicate copy of this sheet is enclosed.

NOTE: Where an appropriate time limit under 37 CFR 1.494 or 1.495 has not been met, a petition to revive (37 CFR 1.137(a) or (b)) must be filed and granted to restore the application to pending status.

SEND ALL CORRESPONDENCE TO:

TERESA STANEK REA
BURNS, DOANE, SWECKER & MATHIS, L.L.P.
P.O. Box 1404
Alexandria, Virginia 22313-1404
(703) 836-6620

SIGNATURE

TERESA STANEK REA
NAME

30,427
REGISTRATION NUMBER

Patent
Attorney's Docket No. 016800-464

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Patent Application of	)	
André ROUGIER et al.	)	Group Art Unit: UNASSIGNED
Application No.: UNASSIGNED (Corresponding to PCT/FR00/00561)	)	Examiner: UNASSIGNED
Filed: SEPTEMBER 24, 2001	)	
For: USE OF VITAMIN C OR THE LIKE FOR STIMULATING SKIN CELL SYNTHESIS	)	

PRELIMINARY AMENDMENT

Assistant Commissioner for Patents
Washington, D.C. 20231

Sir:

Prior to examination, kindly amend the above-identified application as follows:

IN THE CLAIMS:

Cancel claims 1-11 without prejudice or disclaimer and insert the following in lieu thereof:

--12. A method for increasing the level of differentiation of skin fibroblasts, comprising applying to the skin, an effective amount of ascorbic acid or one of its analogues to an individual in need thereof.

13. A method for increasing the level of differentiation of skin keratinocytes, comprising applying to the skin an effective amount of its analogues to an individual in need thereof.

14. A method for stimulating the synthesis of cutaneous vimentin, comprising applying to the skin an effective amount of ascorbic acid or one of its analogues to an individual in need thereof.

15. A method for stimulating the synthesis of cutaneous keratin 10, comprising applying to the skin an effective amount of ascorbic acid or one of its analogues to an individual in need thereof.

16. The method according to claim 12, wherein the ascorbic acid analogues are selected from the group consisting of salts, esters and vigars.

17. The method according to claim 13, wherein the ascorbic acid analogues are selected from the group consisting of salts, esters and vigars.

18. The method according to claim 14, wherein the ascorbic acid analogues are selected from the group consisting of salts, esters and vigars.

19. The method according to claim 15, wherein the ascorbic acid analogues are selected from the group consisting of salts, esters and vigars.

20. The method according to claim 12, wherein the ascorbic acid analogues are selected from the group consisting of sodium ascorbate, magnesium sodium ascorbyl phosphate and the acetic, propionic and palmitic esters thereof and glycosyl ascorbic acid.

21. The method according to claim 13, wherein the ascorbic acid analogues are selected from the group consisting of sodium ascorbate, magnesium sodium ascorbyl phosphate and the acetic, propionic and palmitic esters thereof and glycosyl ascorbic acid.

22. The method according to claim 14, wherein the ascorbic acid analogues are selected from the group consisting of sodium ascorbate, magnesium sodium ascorbyl phosphate and the acetic, propionic and palmitic esters thereof and glycosyl ascorbic acid.

23. The method according to claim 15, wherein the ascorbic acid analogues are selected from the group consisting of sodium ascorbate, magnesium sodium ascorbyl phosphate and the acetic, propionic and palmitic esters thereof and glycosyl ascorbic acid.--

REMARKS

Entry of the foregoing amendment is respectfully requested.

Respectfully submitted,

BURNS, DOANE, SWECKER & MATHIS, L.L.P.

By: _____
Teresa Stanek Rea
Registration No. 30,427

P.O. Box 1404
Alexandria, Virginia 22313-1404
(703) 836-6620

Date: September 24, 2001

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The invention relates to a process for increasing the level of differentiation and/or proliferation of skin fibroblasts and/or for increasing the level of differentiation of skin keratinocytes by applying to the skin a composition comprising an effective amount of ascorbic acid or of at least one of its analogues.

The invention also relates to a process for stimulating the synthesis of cutaneous vimentin by applying to the skin a composition comprising an effective amount of ascorbic acid or of at least one of its analogues. The invention also relates to a process for stimulating the synthesis of cutaneous keratin 10 by applying to the skin a composition comprising an effective amount of ascorbic acid or of at least one of its analogues.

Human skin consists of two compartments, namely a superficial compartment, the epidermis, and a deep compartment, the dermis.

Natural human epidermis is composed mainly of three types of cells: keratinocytes, which form the great majority, melanocytes and Langerhans cells. Each of these cell types contributes, by virtue of its intrinsic functions, towards the essential role played in the body by the skin, in particular the role of protecting the body against external attack (climate, ultraviolet rays, tobacco, etc.), which is referred to

as the "barrier function". Poor renewal of these cells, and more particularly of the keratinocytes, which is observed in particular with age, leads to poor protection of the skin, which then acquires a dry and/or dull appearance.

The dermis gives the epidermis a solid support. It is also the epidermis' nourishing factor. It consists mainly of fibroblasts and of an extracellular matrix which is itself composed mainly of collagen, elastin and a substance known as ground substance, these components being synthesized by the fibroblasts. Leukocytes, mastocytes and tissue macrophages are also found therein. It also contains blood vessels and nerve fibres.

Vimentin fibres are found extensively in the dermis, since they correspond to the intermediary filament of fibroblasts. These vimentin fibres are also present in melanocytes and in Langerhans cells of the epidermis, and can also be present in keratinocytes when they are in a hyperproliferative state.

Keratins are the intermediary filaments of epithelial cells, such as the keratinocytes in the skin. Thus, four types of keratin exist in the epidermis, including keratin 10, referred to as K10, which is specific for the state of differentiation of the keratinocytes.

With age, the quality of the skin diminishes, and in particular thinning of the dermis is observed. It is also accepted that extrinsic factors such as ultraviolet rays, tobacco or certain treatments (glucocorticoids, vitamin D and derivatives, for example) also have a negative effect on the skin.

The importance of cell renewal and the quality of this renewal can thus be appreciated, both at the epidermal level and at the dermal level, in order thus to be able to combat extrinsic attack which damages the skin, in particular by reducing its barrier function, and to combat the signs of ageing of the skin, whether this is chronobiological or light-induced ageing.

One of the aims of the present invention is thus to increase the level of differentiation and/or proliferation of skin fibroblasts and/or to increase the level of differentiation of skin keratinocytes in order thus to combat extrinsic attack, whether this is physical or chemical attack, which damages the skin, in particular by reducing its barrier function, and to combat ageing of the skin, whether this is chronobiological or light-induced ageing.

The Applicant has now discovered that ascorbic acid applied topically to the skin increases the level of differentiation and/or proliferation of skin fibroblasts and/or increases the level of

differentiation of skin keratinocytes, by applying to the skin a composition comprising an effective amount of ascorbic acid or at least one of its analogues.

Ascorbic acid (or vitamin C) is known to stimulate collagen synthesis, by preventing, in the capacity of a co-factor, the self-inactivation of lysine hydroxylase and proline hydroxylase enzymes and by increasing the synthesis of procollagen mRNAs. Ascorbic acid (or vitamin C) is also known to stimulate the synthesis of skin elastin. Mention may be made in this respect of US patents 5 801 192 and 4 983 382 and patent EP 0 717 983. Mention may also be made of an article entitled "Pola to incorporate vitamin C in new cosmetics line for skin care" in the Japan Economic Journal of 5 June 1984 (page 15). Thus, it has been described that ascorbic acid used in cosmetic compositions makes it possible in particular to treat wrinkles (Fragrance Journal, Vol. 8, No. 6(45) (1980) pp. 38-43, "Cosmetic and vitamin - action and safety to dermatology").

One subject of the invention is thus the use of an effective amount of ascorbic acid or of one of its analogues in a composition or in the preparation of a composition intended to be applied to the skin to increase the level of differentiation and/or proliferation of skin fibroblasts and/or to increase the level of differentiation of skin keratinocytes.

A second subject of the invention is the use of an effective amount of ascorbic acid or of one of its analogues in a composition or in the preparation of a composition intended to be applied to the skin to stimulate the synthesis of cutaneous vimentin.

A third subject of the invention is the use of an effective amount of ascorbic acid or of one of its analogues in a composition or in the preparation of a composition intended to be applied to the skin to stimulate the synthesis of cutaneous keratin 10.

A subject of the invention is also a process for increasing the level of differentiation and/or proliferation of skin fibroblasts in an individual with an abnormally low level of fibroblast differentiation and/or proliferation, comprising the topical application to the skin of an effective amount of ascorbic acid or of one of its analogues.

A subject of the invention is also a process for increasing the level of differentiation of skin keratinocytes in an individual with an abnormally low level of keratinocyte differentiation, comprising the topical application to the skin of an effective amount of ascorbic acid or of one of its analogues.

Specifically, the Applicant has discovered that ascorbic acid applied topically to the skin increases the synthesis of vimentin mRNA and thus increases the level of vimentin synthesis. It has also

discovered that ascorbic acid applied topically to the skin increases the synthesis of keratin 10 mRNA and thus increases the level of synthesis of keratin 10.

These proteins, which are intermediary filaments of skin cells, are thus representative of the proliferative and/or differentiating state of skin cells, more particularly of the cells of the dermis and the epidermis. More particularly, vimentin, which is the intermediary filament of fibroblasts, is representative of the proliferative and/or differentiating state of fibroblasts, and keratin 10 is representative of the differentiating state of keratinocytes.

Thus, by topical application of an effective amount of ascorbic acid or its analogues, these skin cells are renewed more rapidly, the appearance of the skin is improved, the skin is more radiant, less dull, firmer, has better tone and is more elastic, wrinkles are attenuated or their appearance is delayed, and the signs of ageing of the skin are diminished.

Advantageously, the ratio of the synthesis of vimentin mRNAs to that of keratin 10 due to the topical application of ascorbic acid is comparable to that without topical application of ascorbic acid. This indicates that the state of the skin, after topical application of ascorbic acid, is maintained in a normal state (without, for example, hyperproliferation or

hyperdifferentiation of one dermal or epidermal compartment relative to the other).

The ascorbic acid analogues are, more particularly, its salts, especially such as sodium ascorbate and magnesium or sodium ascorbylphosphate, its esters, especially such as its acetic, propionic or palmitic esters, or its sugars, especially such as glycosyl ascorbic acid.

The ascorbic acid is generally in L form, since it is usually extracted from natural products.

The effective amount of ascorbic acid or its analogues which can be used according to the invention is, of course, that which is necessary to obtain the expected effects according to the invention. To give an order of magnitude, this amount preferably represents from 0.001% to 20% of the total weight of the composition, preferably from 0.1% to 15% of the total weight of the composition and advantageously from 3% to 10% of the total weight of the composition.

In addition, the composition of the invention is used for a period which is sufficient to obtain the expected effects according to the invention. To given an order of magnitude, this duration can be a minimum of 15 days, but can also be more than 4 weeks, or even more than 8 weeks.

The composition of the invention which is intended for topical application contains a

physiologically acceptable medium, that is to say a medium which is compatible with the skin, including the scalp, mucous membranes and/or the eyes, and can in particular constitute a cosmetic or dermatological composition.

This composition can be in any pharmaceutical form normally used in cosmetics and dermatology, and it can especially be in the form of an aqueous solution which may be in gelled form, a dispersion of the lotion type which may be in two-phase form, an emulsion obtained by dispersing a fatty phase in an aqueous phase (O/W) or, conversely, (W/O), or a triple emulsion (W/O/W or O/W/O) or a vesicular dispersion of ionic and/or nonionic type. These compositions are prepared according to the usual methods.

The composition of the invention can consist, for example, of a lotion, a gel, a cream or a milk and, for example, a make-up-removing or cleansing lotion or milk, a shampoo or a shower gel.

The example which follows illustrates the invention without limiting it in any way. In the compositions, the proportions indicated are percentages by weight, except where otherwise indicated.

Example:

1. Method

10 women aged between 55 and 60 applied, to the lower neck, once a day for 3 months, on one side a water-in-oil emulsion (vehicle or placebo), and on the other side the same water-in-oil emulsion but also comprising 5% vitamin C (= composition or active agent).

Composition:

L-Ascorbic acid	5.00%
Sodium hydroxide	1.83%
Citric acid monohydrate	1.24%
Disodium EDTA	0.05%
Apricot kernel oil	3.00%
Silicone oil	4%
Cyclopentasiloxane and dimethicone copolyol	20%
Dimethicone and dimethiconol	3%
Glycerol	23%
Propylene glycol	4%
Fillers	7%
Preserving agents	0.30%
Water	qs 100.00%

Biopsies of these treated areas were then taken.

2. Extraction and purification of the total RNAs

The biopsies are ground under liquid nitrogen in a Mikrodismembrator S (Braun). The powder obtained is collected in a Teflon capsule with 2 ml of lysis solution (5M guanidine isothiocyanate, 0.1M mercaptoethanol, 0.017M sodium laurylsucosyl, 0.025M sodium citrate pH7, 3 µl/ml antifoam). The suspension is transferred into a tube and is shaken at room temperature for 15 minutes. The lysate is deposited on the surface of a cushion of 1.4 ml of 5.7M caesium chloride, 0.1M EDTA pH 7 in a 3.8 ml polyallomer tube for an SW60 rotor (Beckman L70M Ultracentrifuge). Ultracentrifugation is carried out at 35,000 rpm for 18 hours at 20°C. The pellet is rinsed with absolute ethanol, centrifuged at 13,000 rpm at 4°C for 10 minutes and dissolved in 100 µl of distilled water.

3. Quantification of the concentration of total RNA and of specific mRNA

The amount of RNA collected from the biopsies is estimated from the optical density of the solution at 260 nm, and is then measured by amplifying the ribosomal 28S RNA by RT-PCR. The specific mRNAs are measured by quantitative RT-PCR on aliquots of the same dilution of total RNA, stored at -80°C until the time of use.

Measurement of the mRNA of keratin 10 (K10) and of vimentin

The oligonucleotide primers specific for the genes studied comprise 24 bases, have an A-T percentage of close to 50 and are chosen on two different exons in order to avoid amplification of any traces of DNA present in the samples. The optimum amplification conditions (temperature and number of cycles) were determined for each of the genes studied, taking into account their level of expression in the skin. The RT-PCR is carried out using the Gene Amp rTth kit from Perkin Elmer or the Titam kit from Boehringer.

Each RT-PCR reaction is carried out in the presence of a known number of copies of a synthetic RNA created in the laboratory, containing the oligonucleotide primer sequences specific for the mRNAs of interest and whose amplification product has a molecular size which distinguishes it from the endogenous mRNA. This multistandard makes it possible to control and calculate the yield for the reverse transcription and the amplification reaction.

The amplification products are analysed by polyacrylamide gel electrophoresis followed by staining with CyberGreen. The intensity of the fluorescent signals is measured using a Fluoro S MultiImager. The results are corrected for the RT-PCR yield and are

expressed in arbitrary units per unit of ribosomal 28S RNA.

4. Statistical analysis

The statistical analysis was carried out using the unilateral Student t Test on the ratios of the Active agent (vitamin C)/Placebo (= A/P) values.

$$t(n-1) = \frac{(M\ A/P - 1)Vn}{M\ \text{standard deviations}}$$

For a degree of freedom n-1 = 9, the A/P ratio is significantly greater than 1 with a probability of greater than 95% for a value of $t > 1.83$ and a probability of greater than 99% for a value of $t > 2.82$.

5. Results

Measurement of the total RNA obtained from the biopsies

The total amount of RNA purified from the biopsies is evaluated in a first stage by measuring the optical density at 260 nm and their quality is estimated by measuring the 260/290 nm OD ratio.

Largely sufficient amounts of RNA were obtained from each of the biopsies (between 2.1 and 6.3 µg) with a satisfactory degree of purity (260/280 OD ratio).

The concentration of total RNA is brought by dilution to a calculated value of 4 nanograms per µl. This process allows the reverse transcription and amplification reactions to be carried out on similar amounts of total RNA for all the samples. The amount of total RNA present in the dilute solution is determined quantitatively by measuring the ribosomal 28S RNA, this being carried out in triplicate.

This same RNA solution will be used for all the measurements of the specific RNAs, the results of which are expressed in 28S RNA units.

<u>Measurement of the equilibrium level of the vimentin and keratin 10 mRNAs</u>

The results expressed in arbitrary units per unit of 28S RNA are detailed in Tables 1 and 2.

Table 1: Vimentin mRNA

Subject	Active agent	Placebo	A/P
a	96.7	51.4	1.88
b	70.9	55.0	1.29
c	67.5	77.7	0.87
d	200.3	131.1	1.53
e	123.0	91.1	1.35
f	106.0	102.5	1.03
g	98.5	92.5	1.06
h	81.4	98.6	0.83
i	112.9	128.6	0.88
j	81.7	66.1	1.24
Average	103.9	89.5	1.20*
Standard deviation	38.4	27.6	0.33

Unilateral Student test: *t = 2.02, P<0.05

Seven out of 10 subjects show an equilibrium level of vimentin mRNA which is increased by ascorbic acid.

Table 2: Keratin 10 (K10) mRNA

Subject	Active agent	Placebo	A/P
a	13.4	8.5	1.58
b	10.3	3.9	2.64
c	12.8	13.8	0.93
d	44.2	48.9	0.90
e	25.5	21.8	1.17
f	40.0	23.8	1.68
g	27.4	14.6	1.88
h	21.6	18.4	1.17
i	35.2	28.7	1.23
j	19.5	13.4	1.46
Average	25.0	19.6	1.46**
Standard deviation	11.8	12.6	0.52

Unilateral Student test: **t = 2.95, $p<0.01$

Eight out of 10 subjects show an equilibrium level of keratin 10 mRNA which is increased with ascorbic acid.

The vimentin/keratin 10 ratio is detailed in Table 3.

Table 3: Vimentin/keratin 10 (VIM/K10) ratio

	VIM / K10	
Subject	Active agent	Placebo
a	7.22	6.05
b	6.88	[14.10]
c	5.27	5.63
d	4.53	2.68
e	4.82	4.18
f	2.65	4.31
g	3.59	6.34
h	3.77	5.36
I	3.21	4.48
j	4.19	4.93
Average	4.61	4.88

These results indicate that the biopsies contain a proportion of keratin 10 mRNA and vimentin mRNA which is comparable on the side treated with ascorbic acid and the placebo. The results also indicate that the biopsies were taken uniformly from the various individuals. The b- placebo sample is outside the norm.

When the vimentin measurements are compared to the equivalent measurements taken for procollagen I or III mRNA, the average value of these ratios calculated for the series of treated samples and placebo samples is very close, indicating a coordinated modulation of procollagen and vimentin expression. In

addition, if it is considered that vimentin is representative of the dermal compartment, since it is the intermediary filament of fibroblasts, then increasing the expression of procollagens I and III is accompanied by a parallel increase in vimentin expression and suggests that ascorbic acid either induces an increase in the number of connective cells in the dermis or induces activation of their biosynthetic phenotype.

CLAIMS

1. Use of an effective amount of ascorbic acid or of one of its analogues in the preparation of a composition intended to be applied to the skin to increase the level of differentiation ~~and/or proliferation~~ of skin fibroblasts.

2. Use of an effective amount of ascorbic acid or of one of its analogues in the preparation of a composition intended to be applied to the skin to increase the level of differentiation of skin keratinocytes.

3. Use of an effective amount of ascorbic acid or of one of its analogues in a composition or in the preparation of a composition intended to be applied to the skin to stimulate the synthesis of cutaneous vimentin.

4. Use of an effective amount of ascorbic acid or of one of its analogues in a composition or in the preparation of a composition intended to be applied to the skin to stimulate the synthesis of cutaneous keratin 10.

5. Use according to one of the preceding claims, characterized in that the ascorbic acid analogues are chosen from its salts, its esters and its sugars.

6. Use according to the preceding claim, characterized in that the ascorbic acid analogues are

chosen from sodium ascorbate, magnesium sodium ascorbyl phosphate, its acetic, propionic and palmitic esters, and glycosyl ascorbic acid.

7. Use according to any one of the preceding claims, characterized in that the amount of ascorbic acid or its analogues represents from 0.001% to 20%, preferably from 0.1% to 15% and advantageously from 3% to 10%, relative to the total weight of the composition.

8. Process for increasing the synthesis of cutaneous vimentin in an individual who is deficient in cutaneous vimentin, comprising the topical application to the skin of an effective amount of ascorbic acid or of one of its analogues.

9. Process for increasing the synthesis of cutaneous keratin 10 in an individual who is deficient in cutaneous keratin 10, comprising the topical application to the skin of an effective amount of ascorbic acid or of one of its analogues.

10. Process for increasing the level of differentiation ~~and/or proliferation~~ of skin fibroblasts in an individual with an abnormally low level of fibroblast differentiation ~~and/or proliferation~~, comprising the topical application to the skin of an effective amount of ascorbic acid or of one of its analogues.

11. Process for increasing the level of differentiation of skin keratinocytes in an individual with an abnormally low level of keratinocyte differentiation, comprising the topical application to the skin of an effective amount of ascorbic acid or of one of its analogues.

PCT ORGANISATION MONDIALE DE LA PROPRIETE INTELLECTUELLE
Bureau international

DEMANDE INTERNATIONALE PUBLIEE EN VERTU DU TRAITE DE COOPERATION EN MATIERE DE BREVETS (PCT)

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(71) Déposant *(pour tous les Etats désignés sauf US)*: LA ROCHE POSAY LABORATOIRE PHARMACEUTIQUE [FR/FR]; 3–7, avenue Sainte–Anne, Boîte postale 224, F–92602 Anières (FR).

(72) Inventeurs; et

(75) Inventeurs/Déposants *(US seulement)*: ROUGIER, André [FR/FR]; 13, chemin de l'Isle, F–95550 Bessancourt (FR). RICHARD, Alain [FR/FR]; 68, avenue Géo André, F–44600 Saint–Nazaire (FR).

(74) Mandataire: RENARD, Emmanuelle; L'Oréal – DPI, 6, rue Bertrand Sincholle, F–92585 Clichy Cedex (FR).

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(54) Title: USE OF VITAMIN C OR THE LIKE FOR STIMULATING SKIN CELL SYNTHESIS

(54) Titre: UTILISATION DE LA VITAMINE C OU ANALOGUES POUR STIMULER LA SYNTHESE DE CELLULES DE LA PEAU

(57) Abstract

The invention concerns a method for increasing the differentiation and/or proliferation rate of skin fibroblasts and/or increasing the differentiation rate of skin keratinocytes by applying on the skin a composition comprising an efficient amount of ascorbic acid or of at least one of its analogues. The invention also concerns a method for stimulating cutaneous vimentin by applying on the skin a composition comprising an efficient amount of ascorbic acid or of at least one of its analogues. The invention further concerns a method for stimulating cutaneous keratin 10 synthesis by applying on the skin a composition comprising an efficient amount of ascorbic acid or of at least one of its analogues.

(57) Abrégé

L'invention se rapporte à un procédé pour augmenter le taux de différenciation et/ou de prolifération des fibroblastes de la peau et/ou augmenter le taux de différenciation des kératinocytes de la peau en appliquant sur la peau une composition comprenant une quantité efficace d'acide ascorbique ou d'au moins un des ses analogues. Elle a également trait à un procédé pour stimuler la synthèse de la vimentine cutanée en appliquant sur la peau une composition comprenant une quantité efficace d'acide ascorbique ou d'au moins un des ses analogues. Elle a en outre trait à un procédé pour stimuler la synthèse de la kératine 10 cutanée en appliquant sur la peau une composition comprenant une quantité efficace d'acide ascorbique ou d'au moins un de ses analogues.

OA 99094/45

MAR 0 5 2002

COMBINED DECLARATION FOR PATENT APPLICATION AND POWER OF ATTORNEY (Includes Reference to Provisional and PCT International Applications)	Attorney's Docket No. 016800-464

As a below named inventor, I hereby declare that:
My residence, post office address and citizenship are as stated below next to my name;
I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled:

USE OF VITAMIN C OR THE LIKE FOR STIMULATING SKIN CELL SYNTHESIS

the specification of which (check only one item below):

☐ is attached hereto.

☐ was filed as United States application
Number _____
on _____
and was amended
on _____ (if applicable).

☒ was filed as PCT international application
Number PCT/FR00/00561
on 7 March 2000
and was amended
on _____ (if applicable).

I hereby state that I have reviewed and understand the contents of the above-identified specification, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose to the Office all information known to me to be material to patentability as defined in Title 37, Code of Federal Regulations, §1.56.

I hereby claim foreign priority benefits under Title 35, United States Code, §119 (a)-(e) of any foreign application(s) for patent or inventor's certificate or of any PCT international application(s) designating at least one country other than the United States of America listed below and have also identified below any foreign application(s) for patent or inventor's certificate or any PCT international application(s) designating at least one country other than the United States of America filed by me on the same subject matter having a filing date before that of the application(s) of which priority is claimed:

PRIOR FOREIGN/PCT APPLICATION(S) AND ANY PRIORITY CLAIMS UNDER 35 U.S.C. §119:

COUNTRY (if PCT, indicate "PCT")	APPLICATION NUMBER	DATE OF FILING (day, month, year)	PRIORITY CLAIMED UNDER 35 U.S.C. §119
France	99/03675	24 March 1999	X Yes _No
			_Yes _No
			_Yes _No
			_Yes _No
			_Yes _No

(05/01)

COMBINED DECLARATION FOR PATENT APPLICATION AND POWER OF ATTORNEY (CONT'D) (Includes Reference to Provisional and PCT International Applications)	Attorney's Docket No. 016800-464

I hereby claim the benefit under Title 35, United States Code § 119(e) of any United States provisional application(s) listed below.

(Application Number) (Filing Date)

(Application Number) (Filing Date)

I hereby claim the benefit under Title 35, United States Code, §120 of any United States applications(s) or PCT international application(s) designating the United States of America that is/are listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in that/those prior application(s) in the manner provided by the first paragraph of Title 35, United States Code, §112, I acknowledge the duty to disclose to the Office all information known to me to be material to the patentability as defined in Title 37, Code of Federal Regulations §1.56, which became available between the filing date of the prior application(s) and the national or PCT international filing date of this application:

PRIOR U.S. APPLICATIONS OR PCT INTERNATIONAL APPLICATIONS DESIGNATING THE U.S. FOR BENEFIT UNDER 35 U.S.C. §120:

U.S. APPLICATIONS		STATUS *(check one)*		
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PCT APPLICATION NO.	PCT FILING DATE	U.S. APPLICATION NUMBERS ASSIGNED (if any)			

COMBINED DECLARATION FOR PATENT APPLICATION AND POWER OF ATTORNEY (CONT'D) (Includes Reference to Provisional and PCT International Applications)	Attorney's Docket No. 016800-464

I hereby appoint the following attorneys and agent(s) to prosecute said application and to transact all business in the Patent and Trademark Office connected therewith and to file, prosecute and to transact all business in connection with international applications directed to said invention:

William L. Mathis	17,337	Eric H. Weisblatt	30,505	Bruce T. Wieder	33,815
Robert S. Swecker	19,885	James W. Peterson	26,057	Todd R. Walters	34,040
Platon N. Mandros	22,124	Teresa Stanek Rea	30,427	Ronni S. Jillions	31,979
Benton S. Duffett, Jr.	22,030	Robert E. Krebs	25,885	Harold R. Brown III	36,341
Norman H. Stepno	22,716	William C. Rowland	30,888	Allen R. Baum	36,086
Ronald L. Grudziecki	24,970	T. Gene Dillahunty	25,423	Brian P. O'Shaughnessy	32,747
Frederick G. Michaud, Jr.	26,003	Patrick C. Keane	32,858	~~Kenneth B. Leffler~~	~~36,075~~
~~Alan E. Kopecki~~	25,813	~~B. Jefferson Boggs, Jr.~~	~~32,344~~	Fred W. Hathaway	32,236
Regis E. Slutter	26,999	William H. Benz	25,952	Wendi L. Weinstein	34,456
Samuel C. Miller, III	27,360	Peter K. Skiff	31,917	Mary Ann Dillahunty	34,576
Robert G. Mukai	28,531	Richard J. McGrath	29,195		
George A. Hovanec, Jr.	28,223	Matthew L. Schneider	32,814		
James A. LaBarre	28,632	Michael G. Savage	32,596		
E. Joseph Gess	28,510	Gerald F. Swiss	30,113		
R. Danny Huntington	27,903	Charles F. Wieland III	33,096		

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and: ____________________

Address all correspondence to:

21839

Norman H. Stepno
BURNS, DOANE, SWECKER & MATHIS, L.L.P.
P.O. Box 1404
Alexandria, Virginia 22313-1404

Address all telephone calls to: Norman H. Stepno at (703) 836-6620.

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

FULL NAME OF SOLE OR FIRST INVENTOR	SIGNATURE	DATE
André Rougier	André Rougier	01/03/02
RESIDENCE: 13, Chemin de l'Isle, F-95550 Bessancourt, FRANCE		CITIZENSHIP: FR
POST OFFICE ADDRESS: 13, Chemin de l'Isle, F-95550 Bessancourt, FRANCE		
FULL NAME OF SECOND JOINT INVENTOR, IF ANY	SIGNATURE	DATE
Alain Richard	Alain Richard	01/03/2002
RESIDENCE: 68, Avenue Géo André, F-44600 Saint-Nazaire, FRANCE		CITIZENSHIP: FR
POST OFFICE ADDRESS: 68, Avenue Géo André, F-44600 Saint-Nazaire, FRANCE		
FULL NAME OF THIRD JOINT INVENTOR, IF ANY	SIGNATURE	DATE
RESIDENCE		CITIZENSHIP
POST OFFICE ADDRESS		

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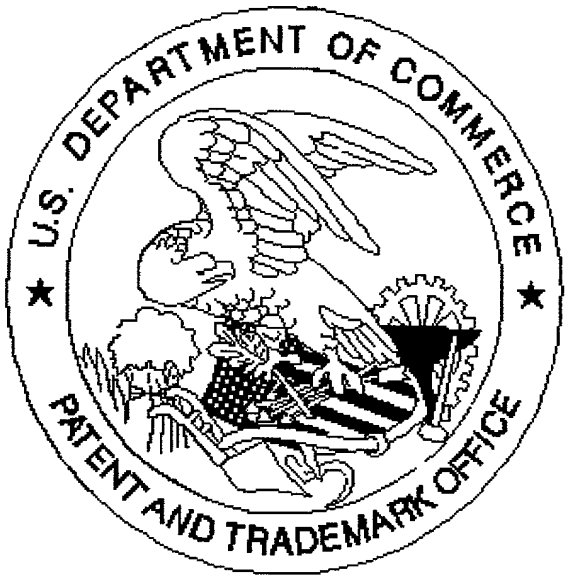

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